A Portable Setup for Molecular Detection by Transmission LSPR

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ABSTRACT

In the framework of bioanalytics and multiple array detection, we developed a fully portable and low-cost detection system based on Localized Surface Plasmon Resonance (LSPR) in a transmission configuration (T-LSPR). The transmission approach is suitable to be scaled to small dimension systems and to enable high-density array measurements on the same platform. Our setup is made out of off-the-shelf components and consists of a set of discrete light sources and a couple of light-detectors which enable a differential measurement setup. An algorithm fits the measured data and extracts the information of the plasmon peak position in the spectrum. The performance of our T-LSPR measurement system has been characterized on a set of Fluorinated Tin Oxide-coated glass slides covered with gold Nanoislands (NIs). The samples have been modified with a single-stranded DNA layer and a real-time DNA hybridization experiment has been performed. Here we demonstrate that the proposed T-LSPR device, based on the characterization of the plasmon peak with a differential approach, is able to monitor real-time DNA hybridization on surface, and to precisely measure the position of the peak with a standard deviation in wavelength of 0.2 nm.

Keywords: sensor, biological, optical.

INTRODUCTION

Parallel detection of multiple analytes by means of high-throughput self-contained automated analytical systems is at the basis of future diagnostics. T-LSPR [1-4] is a very sensitive label-free technique suitable for arrays implementation [5]. It is based on the excitation of the interface between a dielectric and a non connected pattern of metal, on which a localized surface plasmon wave arises. Surface plasmons are very sensitive to changes occurring in the immediate surroundings of the metal nanoparticles, including molecular binding events on the sensor surface. The presence of biomolecules on surface alters the local refractive index causing a shift of the plasmon towards higher wavelengths [6, 7]. Quantitative characterization of the molecular layer on the surface can be obtained from the location of the peak position in the spectrum. The peak wavelength of the plasmon depends on the metal, on the geometry and on the properties of the surrounding dielectric [8]. The sensitivity of a sensor is proportional to the sharpness of its plasmon, which can be narrowed by enhancing the regularity of the nanostructures and of the patterns [9]. In T-LSPR, the plasmon phenomenon can be observed as

an extinction peak in the transmitted light through the metal pattern. The transmission approach is suitable to be scaled to a compact setup and to perform high-density array measurements.
In the present work, a compact fully-portable detection system based on T-LSPR is presented and its performance in terms of real-time monitoring of binding events is reported. We employed gold NIs [10] functionalized with single-stranded DNA in order to perform the real-time observation of DNA hybridization, and we compared our T-LSPR setup with a high-end commercial SPR system.

EXPERIMENTAL DETAILS

Fabrication of Nanoislands sensors

NIs were fabricated on Fluorine-doped Tin Oxide (FTO) covered glass by direct thermal evaporation and subsequent thermal annealing. Surface preparation consists in 20 minutes sonication in a 1:1 solution of 2-propanol and acetone, rinsing with ultra pure water and drying with pure nitrogen. A layer of 5 nm of gold was deposited on surface at RT at 3×10^{-6} mbar at a rate of 0.0016 nm/sec. Samples were placed in the oven at 200°C overnight. Figure 1 shows the formation of NIs after the evaporation and annealing processes. Stability of gold nanostructures on surface is achieved by the employment of the FTO covered glass rather than plain glass, in fact, the porous structure of FTO allows the penetration of gold during the annealing process [11]. As a result, NIs are stable in wet environment without any further surface treatment needed.

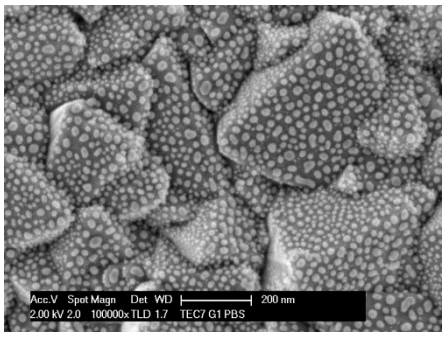

Figure 1. SEM image of gold NIs on FTO covered glass after thermal evaporation and subsequent thermal annealing.

Surface modification of NIs and DNA hybridization

The immobilization of DNA capture single-strands on gold was achieved by sulfur-gold bonds. The NIs FTO-glass slides were incubated at room temperature for 16 hours in Running Buffer (RB; 0.5 M NaCl, 50 mM Na_2HPO_4, pH 6.8) containing the single-stranded DNA (ssDNA) probe. After the incubation, the samples were rinsed with PBS buffer and ultra pure water, and then gently dried with pure nitrogen.
Once the microfluidic was mounted onto the functionalized NIs FTO-glass slides, DNA injections were performed in RB at a flow of 2.5 µl/min for the indicated time. A three-step DNA hybridization protocol was used, which is composed by the subsequent injections of the ssDNA molecules OligoA, OligoB and OligoC.
The sequences of ssDNA used in this work are:
Probe: 5'-CGTACATCTTCTTCCTTTTT-3'-SH (Molecular Weight, MW: 6,132 Da);

OligoA: 5’-AGGAAGAAGATGTACGACCAGCTCAACGAGAAGGTCGCAG-3’ (MW 12,451 Da);
OligoB: 5’-TCAACGAGAAGGTCGCAGTAAGTCCTGCGACCTTCTCGTTGAGCTGGT-3’ (MW 14,804 Da);
OligoC: 5’-GACTTACTGCGACCTTCTCGTTGAACCAGCTCAACGAGAAGGTCGCAG-3’ (MW 14,742 Da).
All the oligonucleotide sequences have been purchased from Sigma-Aldrich, Switzerland.

Microfluidics

Microfluidic channels for the delivery of analytes in liquid samples to the surface are built by mounting a cover plate on top of the NIs FTO-glass slides. Adhesion is obtained thanks to a black double coated tape (High Performance Double Coated Tape 9086, 3M) that proved to adhere on the gold NIs and to be resistant to the injection of aqueous solution. The black color tape screens the contribution of the sensor areas external to the channels. The channels have been patterned by removing material from the tape by laser micromachining; they feature dimensions of 0.8 mm × 8 mm × 150 µm, where the height is defined by the tape thickness.
The cover plate is made of Polymethyl Methacrylate (PMMA), a transparent thermoplastic material frequently used as a substitute of glass. This choice allows us to keep very high transmission properties and high flexibility in design. Inlets and outlets feature a diameter of 0.68 mm, allowing the usage of standard syringe needles. The PMMA cover plate has been designed with two identical channels in order to be able to perform parallel measurements and background noise subtraction. The channels from the NIs sample and from the reference sample are connected in series, as shown in Figure 2, so that the contribution of the bulk solution is canceled out by the differential measurement.

Figure 2. Differential microfluidic system showing the NIs sample (top) and the reference sample (bottom) connected in series.

Transmittance setup

A compact and fully-portable system based on T-LSPR employing electronically-driven power Light Emitting Diodes (LEDs) in the visible range and integrated instrumentation amplifiers is presented. Two parallel photodiodes collect the light passing through the sample and the reference sample, respectively. Our approach is based on the fact that the information of T-LSPR is contained in the position of the plasmon peak in the spectrum. An algorithm fits the data obtained with three LEDs and extracts the peak location. It takes into account the spectral characteristics of all the components, namely, the fact that the LEDs do not emit at a single wavelength and that the photodiodes have responses that change over the visible range. A detailed description of the transmittance setup and the peak extraction algorithm can be found in [12]. The hereby described system is an improvement of the first prototype that includes the

second photodiode to perform real-time differential measurements. The parallel approach allows for light drift and artifacts compensation and it is the first step towards multiple array detection.

RESULTS AND DISCUSSION

NIs stability in aqueous solution

Extinction spectrum of gold NIs on FTO-covered glass slides has been measured prior and after 24 hours immersion in running buffer. Results in Figure 33 show that there is no appreciable shift in the extinction spectrum, demonstrating that the NIs are stable in static conditions. Spectra have been recorded with a commercial spectrophotometer (Ocean Optics 2000+).

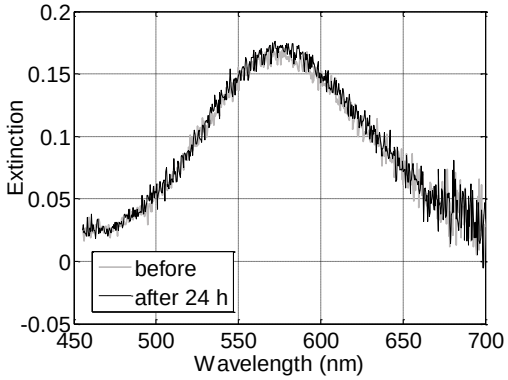

Figure 3. Extinction spectra prior (gray line) and after (black line) 24h NIs immersion in buffer.

NIs adhesion under flow conditions has been tested as well. Microfluidics has been mounted on the NIs sample and the running buffer has been flushed for 10 minutes at 2.5 µl/min. The plasmon peak position has been continuously monitored with our portable setup. The absence of peak-shift proves the stability of gold NIs under flow. In Figure 44 the peak position is represented over time. The standard deviation in the determination of the peak wavelength is 0.2 nm.

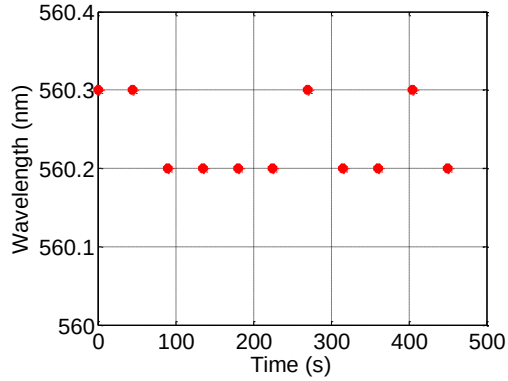

Figure 4. NIs dynamic test: peak position over time recorded for 10 minutes in buffer flow at 2.5 µl/min.

Real-time DNA hybridization

After overnight incubation of gold NIs with Probe DNA, a plasmon peak shift of 6.7 nm has been observed in dry conditions with the spectrophotometer.
Microfluidics has been built on top of ssDNA-functionalized NIs. A three-step DNA hybridization has been performed. At a first step, the OligoA is flushed in the channels. Upon binding with the strand complementary to the Probe DNA immobilized on the surface, OligoA exposes a single-strand trigger complementary to OligoB for the following step (Figure **5**.b). The specific binding leads to a 5.7 nm peak shift measured by means of our portable setup. The second and third steps consist respectively in the binding of OligoB on the single-strand trigger of OligoA (Figure 5.c), and in the binding of OligoC on the single-strand trigger of OligoB (Figure 5.d), bringing an overall additional 5.6 nm peak shift.
The same binding experiment has been run on Biacore X100 in order to compare the kinetics obtained with our portable setup. The same reagents and assay conditions have been used on both systems. Results from the two measurement setups are presented in Figure 5.
The dispersed points in step (a) are due to separate injections of OligoB and OligoC in the absence of OligoA, in order to evaluate the selectivity of these sequences towards the Probe DNA on the surface. The subsequent injection of RB drives the baseline back to its original value, proving that there are no molecules bound to the surface. We assume the bulk effect contribution to be more important in our setup, in contrast with the Biacore instrument, giving rise to the signal observed in step (a). Specific binding of DNA sequences is proved in steps (b), (c) and (d), where an increase in resonance units of the Biacore sensorgram and a peak shift in wavelength for our portable setup are shown. The specificity of the complementary DNA strands has been further verified (data not shown).

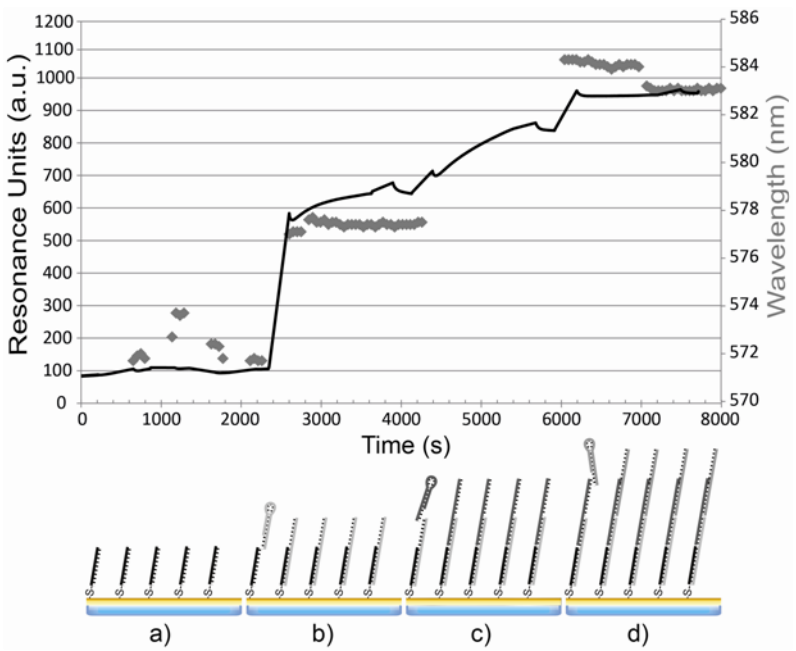

Figure 5. Real-time DNA hybridization experiment. The black line (left axis) represents the SPR measurement from Biacore X100, GE. The gray dots (right axis) represent the peak positions in wavelength as extracted from our setup and algorithm. The following steps are shown: a) buffer baseline with Probe DNA immobilized on the surface. The dispersed points are due to separate

injections of OligoB and OligoC in the absence of OligoA, in order to evaluate the selectivity of these sequences towards the Probe DNA on the surface; b) injection of OligoA; c) injection of OligoB; d) injection of OligoC.

Therefore, we demonstrated here that gold NIs can host heterogeneous DNA hybridization assays. The sensitivity of our sensor can be significantly improved by enhancing the regularity of the nanostructures and surface patterns.

CONCLUSIONS

In this paper we present a fully portable and low-cost detection system based on a differential measurement approach. We developed gold NIs sensors and demonstrate, for the first time, real-time DNA hybridization assays by means of our portable T-LSPR setup. Our setup proves its suitability to observe the real-time binding of molecules on surface. The results, confirmed by the reference experiment performed on a commercially available SPR system, exhibit the same trend for both systems. Implementation of multiple arrays detection together with the further down-scaling of the system, allowed by the usage of low-power components, will make our device fitting the requirements for the diagnostics of the future.

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